Membrane Receptors and Signal Transduction II

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LKB1 is a Critical Regulator of Early Atrial Growth and Electrophysiological Function

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The liver kinase B1 (LKB1)-AMP activated protein kinase (AMPK) pathway regulates cellular metabolism, polarity, and growth. Although previous studies have documented the maladaptive consequences of LKB1 deletion in the progressive development of atrial fibrillation (AF) and cardiac hypertrophy, its role in chamber-specific pathophysiology remains largely unresolved. Therefore we systematically explored LKB1's differential effects on structural and ion channel remodeling in mice with LKB1 deletion in cardiomyocytes. Atrial but not ventricular Nav1.5 and Cx40 expression levels were markedly downregulated in 1d neonates, giving rise to a 2-fold prolongation of the P wave on surface ECG. Detailed ex vivo optical action potential (AP) mapping studies revealed presence of marked inter-atrial conduction abnormalities, loss of bi-atrial electrical coupling, and prolonged AP durations in the atria of mice with LKB1 deletion. In contrast, ventricular changes developed with a much slower time-course that was consistent with previous studies. AMPK α2 inactivated hearts demonstrated modest overlap in ion channel expression, but retained normal ECG characteristics, cardiac structure and function compared to their LKB1 deleted counterparts. LKB1 deletion triggers a primary atrial electrophysiological remodeling program that precedes structural abnormalities and likely underlies the development of AF. LKB1 may be a novel target for the prevention of AF.

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Pathophysiologically-Relevant Levels of Endogenous Cardiotonic Steroids Inhibit the Cardiac Na/K ATPase and Activate ERK1/2 Hypertrophic Signaling In Vivo and In Vitro

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Type-4 cardiorenal syndrome patients develop heart failure as result of kidney failure. While the mechanism of cardiac dysfunction is unknown, elevated levels of circulating endogenous Na/K ATPase inhibitors (NKAi) are detected in patients and animals with kidney failure. We investigated the pathophysiological consequences of NKAi elevation in rat myocytes and hearts of animals with chronic kidney disease (CKD).

Methods: Intracellular Na concentration ([Nai]) was measured using fluorescent ratio-imaging microscopy and ERK1/2 phosphorylation by western blotting, in adult rat ventricular myocytes. Cardiac Na/K ATPase (NKA) activity was measured using biochemical NKA assays. CKD was induced in rats via 5/6 partial-nephrectomy (PN) or in mice via folic-acid administration (FA). Creatinine serum levels were increased in both models, with kidney dysfunction (and cardiac hypertrophy) being more severe in PN model.

Results: Treatment of field-stimulated rat cardiac myocytes with bufalin (10nM) or ouabain (100nM) significantly increased [Nai] by 1.2 ± 0.3 and 1.9 ± 0.2mM, respectively, whereas, concentrations of marinobuganenin below 1μM did not. Furthermore, NKAi activated hypertrophic signaling cascades (assessed by ERK1/2 phosphorylation) with a potency order of bufalin (EC50=70nM)>ouabain (EC50=93nM)> marinobuganenin (EC50=6.4μM). Despite small but significant increases in NKA alpha-1/2 expression, NKA activity in hearts of animals with kidney dysfunction was reduced by $45 \pm 19\%$ in PN (6 weeks post-PN) and $34 \pm 9\%$ in FA animals (12 weeks post FA-injection). NKA inhibition was reversed by incubation with a polyclonal anti-Digoxin antibody (binds most structurally-related NKAi), confirming involvement of NKAi.

Conclusions: Pathophysiologically-relevant concentrations of bufalin and ouabain, but not marinobufagenin, raise [Nai] and activate hypertrophic signaling pathways in the heart, thus contributing to Na and diastolic Ca overload and hypertrophy, during CKD.

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β-Adrenergic Regulation of Cyclic AMP and Ca Current at the T-Tubules and Surface Membrane in Rat Cardiomyocytes

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β-Adrenoceptor (β-AR) signalling is severely impaired in heart failure (HF), and this is accompanied by a loss and disorganization of t-tubules. However, how the latter affects the former is unknown. Here, we examined how acute detubulation affects the β-AR response of L-type Ca^{2+} channel (LTCC) current ($I_{Ca,L}$) and membrane cAMP ($[cAMP]_m$) in adult rat ventricular myocytes (ARVMs) and the contribution of phosphodiesterases PDE3 and PDE4 in this process. ARVMs were infected with an adenovirus encoding a mutant of the olfactory cyclic nucleotide-gated (CNG) channels α subunit to follow $[cAMP]_m$ dynamics by recording the associated cationic current. $[cAMP]_m$ was also monitored by fluorescent imaging using a FRET-based sensor encoding a plasma-membrane targeted cAMP probe (Epac2-camps). Osmotic shock treatment with 1.5M formamide during 15 min induced a loss of t-tubule network as verified by confocal imaging with di-8-ANEPPS staining. Detubulation of ARVMs reduced membrane capacitance by 40% and basal $I_{Ca,L}$ density ~3-fold. Short (15s) applications of isoprenaline (Iso 100nM) increased $I_{Ca,L}$ ~2.5-fold similarly in both conditions but had a 30% smaller effect on $[cAMP]_m$ in detubulated ARVMs. After Iso washout, $I_{Ca,L}$ and $[cAMP]_m$ returned to basal with a ~1.5-fold faster kinetic in detubulated ARVMs than in control, suggesting a faster cAMP degradation by PDEs. The contribution of PDE3 and PDE4 was thus tested using the inhibitors cilostamide (1μM) and Ro20-1724 (10μM), respectively. Both inhibitors had a more pronounced effect on Iso response of $I_{Ca,L}$ and $[cAMP]_m$ in detubulated ARVMs. Thus, detubulation *per se* has a profound effect on the β-AR signalling cascade which may contribute to its impairment in HF. This is partly due to a change in the respective contributions of PDE3 and PDE4 to the hydrolysis of cAMP near the membrane and LTCCs.

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Wnt Signaling Promotes Pacemaker Myocyte Specification of Differentiating Cardiac Progenitor Cells

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Embryonic stem cells (ESCs) can give rise to cardiomyocytes, but mechanisms of cardiac subtype specification to either pacemaker cells or chamber (atrial/ventricular) cardiomyocytes remain little understood. Canonical Wnt signaling plays a key role for ESC maintenance and becomes inactivated during differentiation. We hypothesized that control of Wnt signaling by Dkk1, an endogenous secreted protein inhibitor of canonical Wnt signaling, may impact cardiac subtype specification during ESC differentiation. Mouse ESCs were treated with activin-A and BMP-4 for 40 hours to initiate cardiac differentiation. Flk-1^+/PdgfR-α^+ cardiac progenitors were FACS-purified and seeded as monolayers (day-4). The monolayers were either treated with a saturating level of exogenous Dkk1 (''exo-Dkk1", 150 ng/ml), or cultured in the endogenous level of Dkk1 (''endo-Dkk1", 3.2 ng/ml). At day-8, ~50% of cells were positive for cTnT, a pan-cardiac myocyte marker, in both groups. Endo-Dkk1 group exhibited significantly higher levels of cardiac pacemaker cell markers (Tbx18 and Shox2), but lower levels of chamber lineage markers (Nkx2.5, Isl1, Scn5a and Cacna1c, $p<0.01$). At day-10, endo-Dkk1 group began to beat faster compared to exo-Dkk1 monolayers, and the superior automaticity became more accentuated upon further differentiation (161.5 ± 11.5 vs. 48.0 ± 2.9 bpm, endo- vs. exo-Dkk1, week-3). Endo-Dkk1 monolayers yielded more cells with spontaneous intracellular calcium oscillations compared to exo-Dkk1. Single, spontaneously-beating cells isolated from the endo-Dkk1 monolayers were frequently spindle-shaped, exhibited robust HCN4 proteins and I(f) currents, and fired rhythmic action potentials (288 ± 51 bpm, $n = 5$). Analogous results were attained with two other endogenous Wnt antagonists, Sfrp1 and Sfrp5. Our data demonstrate that the endogenous Wnt pathway of differentiating ESCs promotes specification of cardiac pacemaker cell, rather than chamber cardiomyocytes. Our findings provide a control point to enrich either pacemaker cell or chamber myocyte population.

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Modulation of Adrenergic Signalling by Flavonoids in Cardioprotection

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Dietary flavonoids are thought to contribute to the prevention of cardiovascular disease. Recently we showed that 2,3-dehydrosilybin (DHSB) attenuated